REMARKS

The Pending Claims

Claims 24, 29, and 30 are pending and directed to methods of preparing a creatine amidinohydrolase.

Amendments to the Claims

The claims have been amended to point out more particularly and claim more distinctly the invention. Specifically, claim 24 has been amended to recite the elements of claims 25-28. Accordingly, claims 25-28 have been cancelled to prevent redundancy. Additionally, claim 24 has been amended to recite "Km values for creatine" in step (ii) as suggested by the Office. Claims 29 and 30 are new. Claim 29 is supported by the specification at, for example, column 6, lines 11-16. Claim 30 is similar to claim 24 and is further supported by the specification at, for example, column 9, lines 14-39. No new matter has been added by way of these amendments. The precise changes are recited in Exhibit A.

Summary of the Office Action

The Office requires clarification of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2, because SEQ ID NO: 2 encodes a sequence that differs from SEQ ID NO: 1 at position 145, wherein SEQ ID NO: 2 recites a codon that codes for Asp (yet specifies Glu) and SEQ ID NO: 1 indicates Glu. The Office objects to claim 24, because the phrase "for creatine" is not present after the phrase "Km values" in step (ii). The Office rejects claims 24-28 as allegedly obvious in view of Furukawa et al. (U.S. Patent 5,932,466). Reconsideration is hereby requested.

Discussion of the Substitute Sequence Listing

A substitute sequence listing (paper copy and computer readable copy on a diskette) is enclosed. The Sequence Listing has been amended to correct a typographical error in the nucleotide sequence of SEQ ID NO: 2. The corresponding amino acid sequence disclosed in SEQ ID NO: 1 (and recited in SEQ ID NO: 2) is correct. The correct nucleic acid sequence is evident from the source material, namely the amidinohydrolase gene derived from *Alcaligenes faecalis* TE3581 of deposit accession number FERM P-14237 (see SEQ ID NO: 2, "original source" information; see also column 4, lines 7-10). No new matter has been added by way of this amendment.

Discussion of Claim Objection

Claim 24 has been amended to recite "Km value for creatine" as suggested by the Office. Accordingly, the claim objection is believed to be moot, and Applicants request the withdrawal of the objection.

Discussion of the Obviousness Rejection

The Office contends that Furukawa et al. discloses the amino acid sequence of SEQ ID NO: 2 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2 (namely, SEQ ID NO: 1). The Office alleges that there were several known methods for mutating a protein. The Office argues that Furukawa et al. teaches the desirability of obtaining a creatine amidinohydrolase with a low Km value which provides the motivation to mutate the allegedly known sequence. For these reasons, the Office has rejected the pending claims as obvious in view of Furukawa et al. This rejection is traversed for the following reasons.

Furukawa et al. does not disclose or suggest mutating the amino acid sequence of SEQ ID NO: 2 to arrive at creatine amidinohydrolase with a low Km value. Rather, Furukawa et al. teaches that the creatine amidinohydrolase encoded by SEQ ID NO: 2 is a novel creatine amidinohydrolase that itself has a low Km value (see, e.g., column 1, lines 27-32). In addition to the reported stability at a high pH range, the low Km value of the creatine amidinohydrolase of Furukawa et al. meets the aims set forth in Furukawa et al. (see, e.g., column 1, lines 21-31), such that Furukawa et al. cannot be considered to provide motivation to modify the creatine amidinohydrolase of Furukawa et al. to achieve a lower Km value.

The present inventors were the first to modify the creatine amidinohydrolase of SEQ ID NO: 2 to reduce the Km value. The present invention is based on the selection of a nucleic acid encoding an enzyme derived from *Alcaligenes faecalis* that inherently has a low Km value as a starting material (see claim 24, step (i)) and mutating the starting material to yield an enzyme with an even lower Km value.

Thus, because Furukawa et al. does not teach, suggest, or provide motivation to modify the creatine amidinohydrolase of Furukawa et al. to arrive at an enzyme with an even lower Km value, Furukawa et al. cannot be considered to render obvious the pending claims.

Furthermore, even if one of ordinary skill in the art were motivated to mutate the creatine amininohydrolase of Furukawa et al. in an attempt to yield an enzyme with an even lower Km value, one would not have had a reasonable expectation of success of identifying a creatine amidinohydrolase with a Km value of 3.5-10.0 mM as alleged by the Office. The Office contends that the difference in the presently claimed range of Km values of 3.5-10.0 mM and the Km value of the wild-type enzyme is only slightly higher than experimental

error, citing the wild-type Km value of 13 mM disclosed by Furukawa et al. and the wild-type Km value of 15.2 mM disclosed in the present application.

A possible explanation for the difference in Km values reported by the present application and Furukawa et al. relates to the assays used to determine Km value. In the present application, Km value is measured by a coupling assay containing sarcosine oxidase and peroxidase. In contrast, in Furukawa et al., an assay system containing creatine amidinohydrolase alone is used to determine Km value. Therefore, the method of the pending claims and the method to determine Km value disclosed in Furukawa et al. differ from each other not only in the kind of substrate and the assay buffer used in the assay method, but also in the assay systems themselves.

The Km value is primarily calculated from the results of enzymatic activity measurement at different substrate concentrations (see, e.g., claim 30 and Example 3). In general, a measurement error in the enzymatic activity can be easily suppressed to less than about 5% when one of ordinary skill in the art appropriately performs the assay. Thus, the difference (about 35%) in the Km value between 15.2 mM (wild-type) and 10.0 (claimed) cannot be considered to be only slightly higher than experimental error as argued by the Office.

For the above-stated reasons, one of ordinary skill in the art would not have been motivated nor had a reasonable expectation of success to prepare a creatine amidinohydrolase with a low Km value by mutating the creatine amidinohydrolase of Furukawa et al. Accordingly, the invention as defined by the pending claims is not obvious in view of Furukawa et al., and the obviousness rejection should be withdrawn.

Conclusion

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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